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# Association between Norovirus and Rotavirus Infection and Histo-Blood Group Antigen Types in Vietnamese Children

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**Norovirus and rotavirus are the two most important causes of acute gastroenteritis in children worldwide. Both norovirus and rotavirus recognize human histo-blood group antigens (HBGAs), and multiple binding patterns for HBGAs have been reported. To explore the role of HBGAs in host susceptibility to norovirus and rotavirus, we conducted a cross-sectional study in children hospitalized with diarrhea in northern Vietnam from September 2010 through September 2012. Of 260 children with paired stool and saliva samples, 158 (61%) were classified as HBGA secretors ($Le^{a-b+}$), 31 (12%) were nonsecretors ($Le^{a+b-}$), and 71 (27%) were partial secretors ($Le^{a+b+}$). Norovirus was detected in 50 patients (19%), with viral genotypes GII.3 ($n = 28$) and GII.4 ($n = 22$) being the most common. All children infected with norovirus strains of genotype GII.4 were either HBGA secretors or partial secretors. Of the 28 GII.3 cases, 12 involved HBGA secretors, 11 partial secretors, and 5 nonsecretors. A total of 85 children tested positive for rotavirus, 74 of whom were infected with genotype P[8], 5 with P[4], and 6 with P[6]; all were HBGA secretors or partial secretors. This is the first epidemiological study demonstrating in a population that HBGA phenotype is a key susceptibility factor for both norovirus and rotavirus infections in children.**

Diarrhea remains an important disease with high morbidity and mortality rates, especially among children in developing countries. Enteric viruses such as rotavirus (RV) and norovirus (NoV) play critical roles in severe gastroenteritis in children (1, 2). Before RV vaccine implementation, RV was identified in 40 to 60% of children hospitalized with acute gastroenteritis worldwide, whereas NoV was detected in 3 to 31% of such patients (3–6). In countries in which RV vaccines are used nationwide, NoV is now the main cause of gastroenteritis in children (7, 8). Thus, a successful strategy to control and to prevent acute diarrhea should focus on these two viruses.

Extensive work on the genetics of these viruses has been performed, and both genotypes and genogroups (which include multiple genotypes) have been established. NoVs can be classified into five genogroups, three of which infect humans, i.e., genogroup I (GI), GII, and GIV (9). The most commonly found NoV genogroup is GII, with the GII.4 genotype predominating worldwide. RV classification is currently based on all 11 gene segments of the virus; the genes encoding the spike protein VP4 (P-protease sensitive) and the coat protein VP7 (G-glycoprotein) specify the P and G genotypes, respectively. These proteins are located in the outer capsid of the virus and confer antigenic properties. Group A RVs can be classified into 27 G genotypes and 35 P genotypes, which fall within five P genogroups (10). The most commonly found RV G and P genotypes are G1, G2, G3, G4, P[4], and P[8].

It has been shown that NoV recognizes histo-blood group antigens (HBGAs) and that different NoV genotypes exhibit distinct HBGA binding patterns. For example, eight NoV binding patterns, which are dependent on the virus genotypes and are based on three major binding motifs, in the ABO, secretor (H), and Lewis families of HBGAs, have been described (11). Evidence that HBGA binding plays a role in host susceptibility to NoVs has been obtained from human volunteer challenge studies using the Norwalk virus (12) and a GII.4 NoV (13). The detailed structures of NoV capsid protein interactions with different HBGA oligosaccharides have been resolved using crystallography for a number of representative binding patterns (14–16).

The discovery that RVs recognize human HBGAs as well occurred only recently. Early studies showed that some animal RVs recognize sialic acid as a receptor, but later studies showed that human and most animal RV receptors are sialic acid independent (17, 18). Following earlier research on NoV and HBGAs, Huang et al. demonstrated that the RV surface spike protein VP8* binds to human HBGAs (19). They showed that all three major human P genotypes, P[4], P[8], and P[6], recognize human HBGAs; P[4] and P[8] recognize the Lewis b and H1-type antigens, while P[6] recognizes only the H1-type antigen (19). Binding patterns in another RV genogroup, P[III], have also been characterized recently, revealing even greater diversity. All three genotypes in this genogroup, P[9], P[14], and P[25], recognize A antigens (10). In a separate study, Hu et al. also found that P[14] VP8* recognizes HBGAs (20). These data suggest that HBGAs may play important roles in susceptibility to RV as well as to NoV.

In this cross-sectional study, we performed a survey of children with acute gastroenteritis in a hospital in Vietnam, hypothesizing that the HBGA type would correspond to RV/NoV infection status. We observed a marked relationship between HBGA type and susceptibility to NoV and RV infection. This is the first epidemiological study to corroborate in a population the role of HBGAs in RV susceptibility, in a manner similar to that observed for NoV.

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These data also provide a baseline for future disease control and prevention efforts, including NoV and RV vaccine implementation.

## MATERIALS AND METHODS

**Study population.** The study was conducted in Thai Binh Pediatric Hospital (Thai Binh City, Vietnam). Thai Binh City is situated southeast of the capital city, Hanoi, and features both inland and coastal areas. The population of the city is 1.78 million, and there are 26,639 new births each year (21). The hospital has a total of 200 beds, 45 of which are in the enteric disease department. In this region and nationwide, RV vaccine usage is restricted to the private market; less than 20% of children younger than 6 months of age receive the vaccine (L. T. Luan, personal communication).

Fecal samples were collected from children less than 5 years of age who were hospitalized with acute gastroenteritis between September 2010 and September 2012. All specimens were collected with parental consent. Fecal specimens were collected from children who (i) had experienced diarrhea three or more times within a 24-h period and (ii) had been admitted to the hospital within 7 days after the onset of diarrhea. Trained health care personnel used sterile stool containers to collect fecal samples within 2 days after the admission date. Samples were stored at the hospital at −20°C before being transferred on ice to the National Institute of Hygiene and Epidemiology of Vietnam.

Starting in September 2011, saliva samples were also collected from children. Cotton swabs were used to collect two saliva samples from each child, and the swabs were immediately resuspended in 1 ml phosphate-buffered saline (PBS). The swabs were then removed, and samples were stored at −20°C. Saliva samples were transferred in the same manner as described for fecal samples. Beginning in September 2011, 260 paired saliva-stool samples were collected from children hospitalized with acute gastroenteritis. The study protocol and consent form were approved by the Medical Research Ethical Committee of Vietnam's National Institute of Hygiene and Epidemiology.

**Detection and genotyping of NoV in fecal samples.** The QIAamp viral RNA extraction kit (Qiagen, Hilden, Germany) was used to extract viral RNA from fecal specimens, which were prepared as 20% suspensions in diethylpyrocarbonate (DEPC)-treated water. Subsequently, NoV genogroups GI and GII were detected using two separate real-time reverse transcription (RT)-PCRs targeting the junction of open reading frames 1 and 2. The G1 reaction used primers COG1F and COG1R (1.5 μM) and a 6-carboxyfluorescein (6-FAM)-labeled RING(1a)-TP probe (0.3 μM), while the GII reaction used primers COG2-F1, COG2-F2, and COG2-R (1.5 μM) and a 6-FAM-labeled RING(2)-TP probe (0.1 μM) (Integrated DNA Technologies) (22). The RT-PCR was performed in one step using a Superscript III Platinum one-step quantitative RT-PCR kit (Invitrogen, Carlsbad, CA), on a Q-Rotorgene (Qiagen) platform, under the following conditions: 30 min at 50°C to reverse transcribe the RNA template, followed by 5 min at 95°C and then 45 cycles of 94°C for 20 s and 55°C for 30 s. NoV GII-positive samples with RT-PCR cycle threshold ($C_T$) values of ≤35 were further genotyped using endpoint RT-PCR assays with primer sets G2SKF-G2SKR and COG2F-G2SKR, which target NoV region C (467 and 386 bp, respectively) and Cap D3/Cap C region D (252 bp) (22–24). These primers were used in sequential fashion; that is, if the first primer pair failed to amplify the gene segment, then the second pair was used, and so on. NoV GI-positive samples were subjected to nested RT-PCRs with NV33-NV35 primers followed by NV32-NV36 primers (25). Direct sequencing of the PCR products was attempted for all samples. If it failed, then amplicons were cloned into the pGEM-T vector (Promega), transformed into *Escherichia coli* DH5α, and selected based on ampicillin resistance and blue-white selection with X-Gal and isopropyl-β-thiogalactoside (IPTG). The PCR products or pGEM-T cloned products were sequenced on a 3130xl genetic analyzer (Applied Biosystems). To avoid cross-contamination, separate rooms were used for each step of all molecular procedures. In addition, Milli-Q water and negative extraction controls were used.

Sequence data were analyzed using BioEdit version 7.0.5 (Ibis Bioscience, Carlsbad, CA) and MEGA version 5.0 (26). A NoV genotyping tool (www.rivm.nl/mpf/norovirus/typingtool) was employed, and ClustalW was used to align each sequence with the corresponding sequences from NoV GII.4 and GII.3 strains available in GenBank. Phylogenetic analysis was carried out with MEGA 5.0 using the Kimura 2-parameter model of nucleotide substitution and the neighbor-joining method of tree construction, with 1,000 bootstrap replicates.

**Detection of RV and P and G genotyping.** RV infection was assessed in the same stool samples using Rotaclone, a qualitative enzyme immunoassay (EIA) kit (Meridian, OH). Genotyping of RV-positive samples was carried out using published procedures (27, 28). In brief, RNA samples were reverse transcribed and amplified in a one-step procedure using the primer pair Beg9-End9 for the VP7 gene and the primer pair con2-con3 for the VP4 gene. Subsequently, multiplex nested PCRs were carried out using primers RVG9, aBT1, aCT2, aET3, aDT4, aAT8, and aFT9 for VP7 and primers Humcon5, P[4], P[6], P[8], and P[9] for VP4.

**Determination of ABO and Lewis blood group antigens using saliva samples.** HBGA A, B, O, $Le^a$, $Le^b$, $Le^x$, and $Le^y$ antigenic profiles in children's saliva samples were determined using EIAs and the corresponding monoclonal antibodies against individual HBGAs, as described previously (29, 30). Saliva samples were boiled at 100°C for 10 min, diluted 1:1000 in PBS, and used to coat 96-well plates overnight at 4°C. Internal positive and negative controls consisted of saliva samples with known HBGA phenotypes. After blocking of plates with 5% skim milk in PBS, 100 μl monoclonal antibodies specific for the A, B, and H and Lewis a, b, x, and y antigens (Covance Princeton, New Jersey) were added to each sample and incubated at 37°C for 1 h. Then, peroxidase-conjugated anti-mouse IgG, anti-mouse IgM, or anti-mouse IgG3 was added to the appropriate wells, and the plates were incubated for 1 h at 37°C. After the incubation period, the plates were washed and developed using a tetramethylbenzidine (TMB)-$H_2O_2$ system (KPL). Reactions were stopped after 3 to 5 min, and absorbance at 450 nm/620 nm was measured. The cutoff value for a positive signal was absorbance at 450 nm/620 nm = 0.1.

**Amplification of the NoV VP1 capsid gene.** The complete sequence of open reading frame (ORF) 2 and a partial sequence of ORF 3 containing the P region (including P1 and P2) was amplified using the RING2PCR and PanGIIR1 primer pair in GII.4 (2006b, New Orleans 2009, and Sydney 2012) and GII.3 strains randomly drawn from stool samples with paired saliva samples; this yielded a 2.4-kb product (31). RT-PCR products were sequenced (Macrogen, South Korea) using the original primer pairs and additional internal primers (32). Alignment with NoV reference strains was performed using ClustalW (26).

**Statistical analysis.** Data analysis was performed using SPSS software, version 20.0. Fisher's exact test was used to compare HBGA phenotypes between the group infected with different NoV (or RV) genotypes and the NoV (or RV)-negative group, as well as between groups affected with different viral genotypes. Proportions and 95% confidence intervals (CIs) were calculated. Proportions were compared using chi-square tests.

## RESULTS

**Molecular epidemiology of NoV and RV gastroenteritis.** In this study, 807 stool samples were collected from children hospitalized with acute gastroenteritis at Thai Binh Pediatric Hospital; 400 samples were obtained between September 2010 and August 2011, and 407 samples were obtained between September 2011 and September 2012. Of these 807 samples (Table 1), 346 samples (43%) were positive for NoV ($C_T$ cutoff values of ≤40), and 311 of those samples (90%) had $C_T$ values of ≤35. The NoV detection rate in the first period (from September 2010 through August 2011; 52% [95% CI, 47 to 57%]) was significantly higher ($P < 0.05$) than that in the second period of the study (from September 2011 through September 2012; 34% [95% CI, 30 to 39%]).

NoV was detected throughout the year, but decreases in cases

**TABLE 1** Age distributions of RV and NoV infections in infants and children <5 years of age hospitalized with diarrhea at Thai Binh Pediatric Hospital in Vietnam

| Age (mo) | RV | | NoV | | RV-NoV coinfection | | Total no. of diarrhea cases |
|---|---|---|---|---|---|---|---|
| | No. | % (95% CI)[a] | No. | % (95% CI)[a] | No. | % (95% CI)[a] | |
| <3 | 5 | 19 (8–38) | 4 | 15 (5.5–34) | 0 | 0 | 26 |
| 3–5 | 36 | 29 (22–38) | 51 | 41 (33–50) | 13 | 11 (6–17) | 123 |
| 6–11 | 126 | 38 (33–44) | 149 | 45 (40–51) | 33 | 10 (7.2–14) | 329 |
| 12–23 | 118 | 48 (42–55) | 112 | 46 (40–52) | 34 | 14 (10–19) | 244 |
| 24–35 | 26 | 45 (33–58) | 22 | 38 (27–51) | 6 | 10 (4.5–21) | 58 |
| 36–47 | 7 | 39 (20–61) | 6 | 33 (16–56) | 1 | 5.6 (0–28) | 18 |
| 48–60 | 3 | 33 (12–65) | 2 | 22 (5.3–56) | 0 | 0 | 9 |
| Total | 321 | 40 | 346[b] | 43 | 87 | 11 | 807 |

[a] Percentage (95% CI) of diarrheal infections attributed to each pathogen in each age group.
[b] Samples with $C_T$ values of <40 were considered positive for NoV. Samples with $C_T$ values of ≤35 ($n = 311$) were genotyped (see Table 2).

were evident in January and February, the months in which the RV detection rates were highest (Fig. 1). Interestingly, the two NoV peaks, from October to December 2010 and from June to August 2011, coincided with increased numbers of NoV GII.4 New Orleans 2009 strains. RV was detected in 321 samples (40%), peaking in December through March in both years. There was no difference in RV detection rates in the 2 study periods (39% versus 41%).

The prevalence rates of RV and NoV did not differ significantly between age groups from 6 to 47 months, while the lowest prevalence was found in children less than 3 months of age (Table 1). The majority (~90%) of both NoV and RV infections occurred in children under 2 years of age. Coinfections with NoV and RV occurred in 11% of admissions, with equally high rates in age groups of 3 to 35 months.

**NoV genotypes.** Of the 311 NoV-positive samples with $C_T$ values of ≤35, 287 samples (92%) were successfully genotyped. Genotypes GII.3 (26 to 28%) and GII.4 (55 to 62%) were the most prevalent in this sample during the 2-year surveillance period (Table 2). In 2012, there were fewer GII.4 cases (Fig. 2), although the decrease did not achieve statistical significance; the numbers of GII.3 cases remained constant. In 2012, the prevalence of the GII.4 2006b variant gradually declined, while the prevalence of the New Orleans 2009 variant remained stable. A single case of GII.4 Sydney 2012 was detected in September 2012. Codetection of different genotypes occurred in 0.6% of the NoV-positive samples genotyped. GI.8 and other GII genotypes, such as GII.2, GII.7, GII.12, GII.13, and GII.16, were detected sporadically.

**Circulating RV genotypes.** P[8] was the most common RV genotype, representing 79% (252 cases) of all cases. P[4] (14 cases) and P[6] (19 cases) accounted for 4% and 6% of RV cases, respectively (Table 3). A single case of P[9] was detected. The corresponding genotypes G1, G2, G3, G4, and G9 were commonly observed in this study.

**HBGA phenotypes of NoV-infected children.** Although NoV surveillance began in 2010, the collection of saliva samples was initiated only in the second study period (from September 2011). A total of 260 paired fecal-saliva samples were available for analysis. Of these paired samples, 22 and 28 were found to contain NoV genotypes GII.4 and GII.3, respectively (Table 4). All 22 children infected with GII.4 were either HBGA secretors ($Le^{b+}$ and/or $Le^{y+}$ of H1-type positives) or partial secretors (both $Le^{a+}$ and $Le^{b+}$ or $Le^{x+}$ and $Le^{y+}$); there was not a single case of a nonsecre-

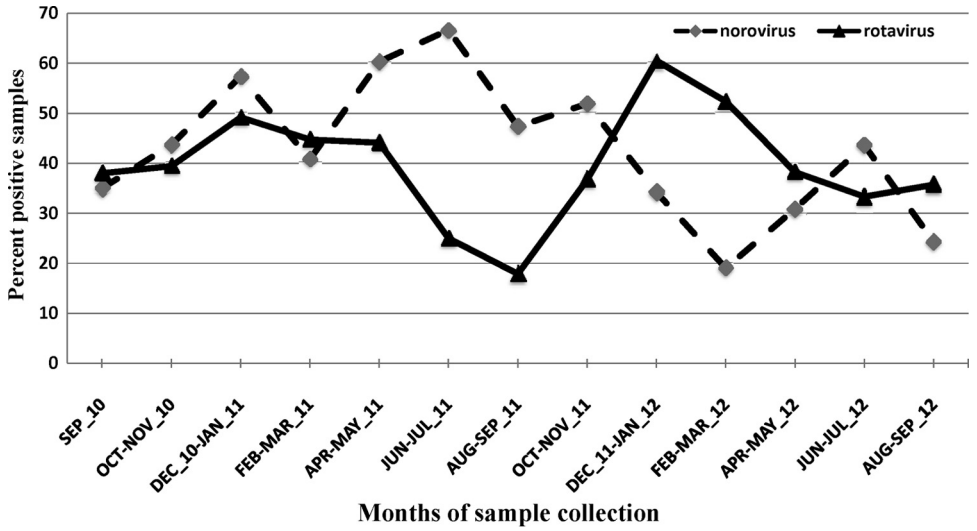


**FIG 1** Monthly distributions of NoV and RV infections in infants and children <5 years of age hospitalized with acute diarrhea at Thai Binh Pediatric Hospital, from September 2010 through September 2012.

**TABLE 2** Distribution of NoV genotypes detected at Thai Binh Pediatric Hospital during a 2-year period of surveillance (2010 to 2012)

| NoV genotype | No. (%) of cases in: | | |
|---|---|---|---|
| | September 2010 to August 2011 | September 2011 to September 2012 | Total |
| GI.8 | 2 (1.0) | 0 (0) | 2 (0.6) |
| GII.2 | 2 (1.0) | 0 (0) | 2 (0.6) |
| GII.3 | 54 (28.1) | 31 (26.1) | 85 (27.3) |
| GII.4 | | | |
| 2006b | 104 (54.2) | 41 (34.5) | 145 (46.6) |
| New Orleans 2009 | 14 (7.3) | 24 (20.2) | 38 (12.2) |
| Sydney 2012 | 0 (0) | 1 (0.8) | 1 (0.3) |
| GII.7 | 1 (0.5) | 0 (0) | 1 (0.3) |
| GII.12 | 1 (0.5) | 0 (0) | 1 (0.3) |
| GII.13 | 6 (3.1) | 2 (1.7) | 8 (2.6) |
| GII.16 | 2 (1.0) | 0 (0) | 2 (0.6) |
| GII.3 + G1.8[a] | 1 (0.5) | 0 (0) | 1 (0.3) |
| GII.3 + GII.4 New Orleans[a] | 1 (0.5) | 0 (0) | 1 (0.3) |
| Not typeable[b] | 4 (2.1) | 20 (16.8) | 24 (7.7) |
| Total NoV samples typed | 192 | 119 | 311 |

[a] Mixed infection.
[b] Genotyping failed with RT-PCR amplification or sequencing.

**TABLE 3** Distribution of RV P and G genotypes at Thai Binh Pediatric Hospital, from September 2010 to September 2012

| G genotype | No. with P genotype of: | | | | |
|---|---|---|---|---|---|
| | P[8] | P[4] | P[6] | P[9] | NT[a] |
| G1 | 233 | 3 | 2 | 0 | 6 |
| G2 | 0 | 8 | 1 | 0 | 1 |
| G3 | 11 | 3 | 3 | 1 | 1 |
| G4 | 1 | 0 | 13 | 0 | 0 |
| G9 | 1 | 0 | 0 | 0 | 0 |
| NT[a] | 6 | 0 | 0 | 0 | 27 |
| Total | 252 | 14 | 19 | 1 | 35 |

[a] NT, not typeable; these samples could not be genotyped.

tor ($Le^{a+b-}$ and/or $Le^{x+y-}$). Among the 28 typed GII.3 cases, 23 were secretors or partial secretors and 5 were nonsecretors.

Most children infected with NoV GII.4 expressed high levels of $Le^b$ and $Le^y$. A few individuals also expressed $Le^a$ and/or $Le^x$ antigens at low levels, indicating an overwhelming prevalence of the secretor phenotype (Fig. 3). The A antigen was coexpressed in 50% of infected individuals. Fifty percent of children infected with NoV GII.3 expressed high levels of $Le^b$ and $Le^y$. The rest, known as partial secretors, coexpressed $Le^a$ and $Le^b$ or $Le^x$ and $Le^y$. A greater percentage of individuals with the partial secretor phenotype were infected with GII.3 than with GII.4.

**HBGA phenotypes of RV-infected children.** All 74 RV P[8]-infected and 5 P[4]-infected individuals were HBGA secretors or partial secretors. By comparison, the nonsecretor phenotype represented 18% of RV-negative cases ($P < 0.001$, compared with RV P[8]) (Table 5). All six P[6] cases involved secretors or partial secretors, although in one case the signals for $Le^a$ and $Le^x$ were stronger than that for $Le^b$. Of note, among 260 children, both RV and NoV were detected in 17 children, all of whom were secretors or partial secretors.

Most children infected with RV P[4] and P[8] expressed $Le^b$ and $Le^y$ (Fig. 4). Low levels of $Le^a$ and $Le^x$ expression (in addition to $Le^b$ and $Le^y$ expression) were detected in a number of children, providing strong confirmation of secretor/partial secretor status. In contrast, $Le^b$ expression levels were low in children infected with RV P[6]. H1 antigen was detected at low levels in all RV-positive individuals.

**Conservation of the HBGA binding domain in Vietnamese GII.4 and GII.3 strains.** A 2.4-kb segment covering the VP1 gene of selected GII.4 and GII.3 variants was amplified and sequenced (Fig. 5A and B). Nucleotide sequence differences of 6 to 9% were observed among the three GII.4 variants, 2006b ($n = 6$), New Orleans 2009 ($n = 3$), and Sydney 2012 ($n = 1$). Amino acid substitutions between Sydney 2012 and New Orleans 2009 (which affected ~3% of nucleotides) occurred in 10 positions across the P2 and P1-P2 regions of the 2.4-kb sequence. The substitutions P(S)294T, A368E (epitope A) (33), S393G (epitope D), and

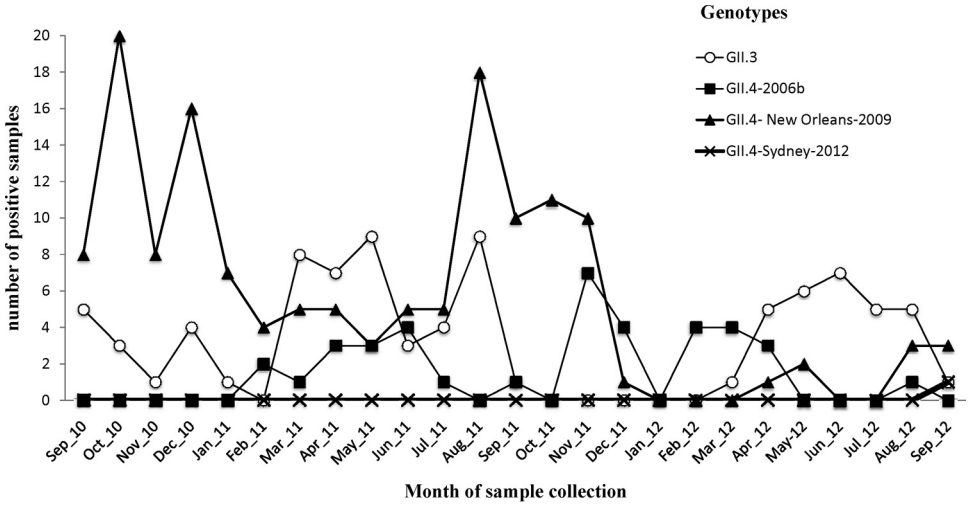


**FIG 2** Monthly distributions of NoV GII.4 and GII.3 genotypes in NoV-positive samples obtained from infants and children hospitalized with acute diarrhea at Thai Binh Pediatric Hospital, from September 2010 through September 2012.

**TABLE 4** Association between NoV infection and host HBGA types among children hospitalized with acute diarrhea at Thai Binh Pediatric Hospital in Vietnam in 2012

| NoV infection status | No. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Total | A | AB | B | O | Secretor ($Le^{b+}$, $Le^{y+}$, or $H^{+}$) | Partial secretor ($Le^{a+b+}$ or $Le^{x+y+}$) | Nonsecretor ($Le^{a+}$ or $Le^{x+}$) |
| NoV-positive | | | | | | | | |
| GII.4 | 22 | 6 | 0 | 3 | 13 | 19 | 3 | 0 |
| GII.3 | 28 | 6 | 1 | 1 | 20 | 12 | 11 | 5[a] |
| NoV-negative | 210 | 49 | 12 | 34 | 115 | 127 | 57 | 26 |
| Total[b] | 260 | 61 | 13 | 38 | 148 | 158 | 71 | 31 |

[a] $P = 0.0588$, compared with the NoV GII.4-infected group, Fisher's exact test.
[b] All children with paired stool-saliva samples were included.

**TABLE 5** Association between RV infection and host HBGA types among children hospitalized with acute diarrhea at Thai Binh Pediatric Hospital in Vietnam in 2012

| RV infection status | No. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Total | A | AB | B | O | Secretor ($Le^{b+}$, $Le^{y+}$, or $H^{+}$) | Partial secretor ($Le^{a+b+}$ or $Le^{x+y+}$) | Nonsecretor ($Le^{a+}$ or $Le^{x+}$) |
| RV-positive | | | | | | | | |
| P[4] | 5 | 1 | 1 | 2 | 1 | 4 | 1 | 0 |
| P[6] | 6 | 1 | 1 | 2 | 2 | 2 | 4 | 0 |
| P[8] | 74 | 20 | 7 | 16 | 31 | 51 | 23 | 0[a] |
| RV-negative | 175 | 39 | 4 | 18 | 114 | 101 | 43 | 31 |
| Total[b] | 260 | 61 | 13 | 38 | 148 | 158 | 71 | 31 |

[a] $P < 0.001$, compared with the RV-negative group, Fisher's exact test.
[b] All children with paired stool-saliva samples were included.

S310D(N) (epitope F) were observed between GII.4 Sydney 2012 and New Orleans 2009. Substitutions in amino acids adjacent to these epitopes (N373H, P396H, and I413T) were also noted between these two variants. Interestingly, despite the extensive sequence variation among the three GII.4 variants, the amino acid motifs in the three HBGA binding sites described by Tan et al. (34) were conserved.

All Vietnamese GII.3 specimens analyzed fell within the GII.3c cluster (Fig. 5B). This cluster showed 3 to 5% amino acid sequence divergence from the GII.3b cluster. The amino acids at binding site positions 356 and 447 were conserved among GII.3 strains isolated from both nonsecretors and secretors. Interestingly, near the second binding site (386D), two amino acid variants (D385G and D385E) were found in strains from nonsecretors, whereas five of the six strains from secretors possessed the D385G substitution.

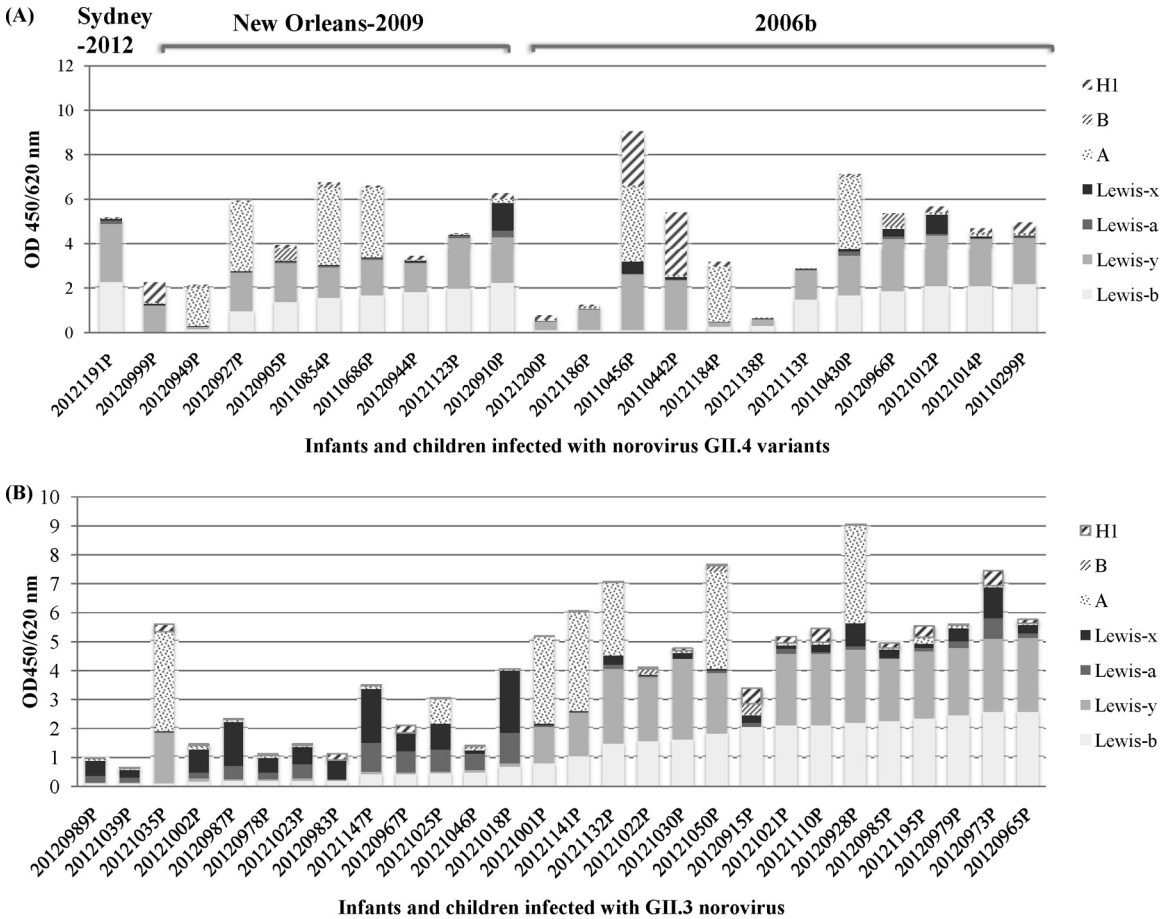


**FIG 3** Profiles of AB and Lewis antigen types in saliva samples from infants and children infected with NoV GII.4 (A) and GII.3 (B) genotypes. Each stacked bar represents a single case. Patient identification numbers are indicated below the bars. OD, optical density.

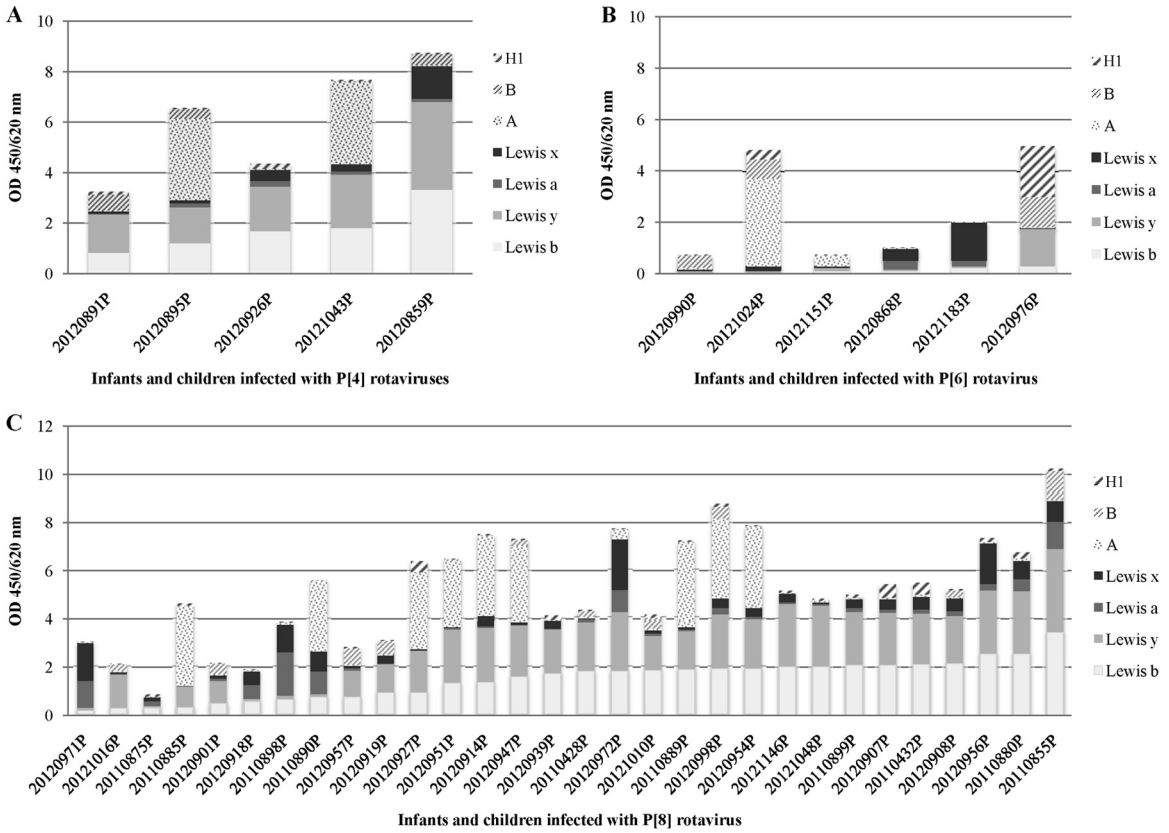


**FIG 4** Profiles of ABH and Lewis antigens in saliva samples from infants and children infected with the three most common RV P genotypes, i.e., P[4] (5 cases) (A), P[6] (6 cases) (B), and P[8] (30 cases) (C). Patient identification numbers are indicated below the bars. OD, optical density.

It remains unknown whether this residue is responsible for differences in binding patterns for host HBGAs.

## DISCUSSION

In this study, we document an association between HBGA secretor status in children and infection with specific RV P genotypes, a finding consistent with the recent observations that RV binds to HBGAs (10, 19, 20). It has been demonstrated that both P[4] and P[8] RVs recognize $Le^b$ and H1-type antigens (19). The fact that both P[4] and P[8] RVs were found only in HBGA secretors or partial secretors strongly suggests that $Le^b$ and H1-type antigens serve as host receptors or attachment factors for these two RV genotypes. Nonsecretors do not express $Le^b$ and H1-type antigens, due to a defect in the *FUT2* gene; they appear to be resistant to P[4] and P[8] RVs.

RV P[6] strains are genetically closely related to the P[4] and P[8] genotypes within the P[II] genogroup. In one of our previous studies, we showed that the VP8* domain of P[6] RVs recognizes only the H1-type antigen (19). In this study, all six P[6] cases involved HBGA secretors or partial secretors. This suggests that P[6] RVs preferentially infect secretors. In our previous binding assays, the H1-type antigen typically gave a weaker signal than most other HBGAs, due to the lower efficiency of the anti-H1 monoclonal antibody used. This can help explain the observation that only 2 saliva samples from P[6]-infected children were of the H1 phenotype. It is interesting that a saliva sample from one of the six P[6]-infected children gave weak signals for all HBGAs; only marginal positive findings were detected for the $Le^a$ and $Le^x$ antigens, and $Le^b$ levels were detectable but below the cutoff value. Thus, this individual was considered a partial secretor rather than a nonsecretor. A future study including more P[6]-infected children is needed to clarify whether P[6] has the ability to bind in nonsecretors.

The preferential infection of secretors by the P[4], P[8], and perhaps P[6] RV genotypes helps explain the predominance of those genotypes in cases of acute gastroenteritis around the world; because most (70 to 80%) of the people in the world are secretors (35, 36), there are many susceptible hosts available to infect. Vietnam is no exception. In the past 15 years, P[4] and P[8] strains have accounted for approximately 90% of RV gastroenteritis cases in children (37–39). Similar to findings reported from Vietnam's RV surveillance program, which was initiated in 1998, we found that P[8] was the most common genotype, representing 79% of all RV cases. Of note, RV vaccines (Rotarix or RotaTeq) have been available in the private market only since 2007 (Rotarix) and 2009 (RotaTeq), and vaccine coverage rates among children 6 to 12 weeks of age have been less than 20% (L. T. Luan, personal communication). Secretor status also appears to be common in Vietnam. In a separate study of HBGA types in Vietnamese children, 86.4% of children hospitalized with diarrhea were secretors, based on *FUT2* genotyping results (N. V. Trang, T. B. H. Vu, T. M. C. Nguyen, and D. D. Anh, unpublished data). It is interesting that the majority of GI and GII NoVs also recognize secretor HBGAs. The same mechanisms of selection and adaptation involving host HBGAs may be important in the evolution of both NoVs and RVs.

In this study, two NoV genotypes, GII.4 and GII.3, were found

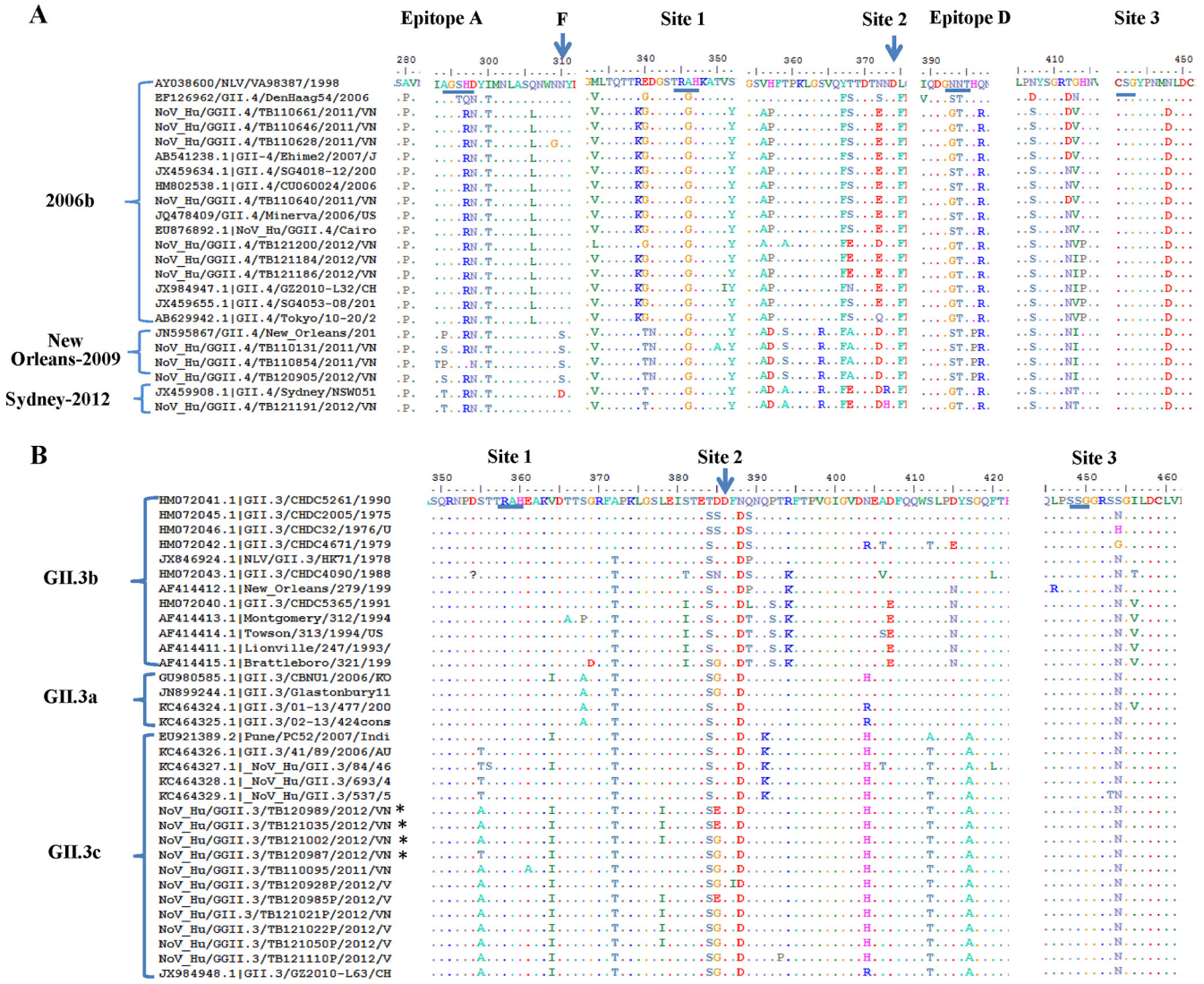


**FIG 5** Sequences of HBGA binding sites in the VP1 capsid region of the GII.4 (A) and GII.3 (B) NoV genotypes. *, GII.3 strains from HBGA nonsecretor hosts. The conserved motifs in the HBGA binding sites (sites 1, 2, and 3, as described by Tan et al. [34]) are underlined. NoV GII.4 blocking epitopes, as described by Debbink et al. (33), are also illustrated.

to predominate, similar to the findings of a previous report on this region (40). Both genotypes primarily infected HBGA secretors, consistent with findings reported by many other groups (30, 33). Collectively, these two genotypes represented 86.1% of the NoV cases in our study. Occasional infections of nonsecretors by NoV GII.4 and GII.3 strains have been reported (35, 41). To our surprise, five children infected with strains belonging to the GII.3c cluster were nonsecretors. Experimental binding of GII.3 virus-like particles (VLPs), representing different strains from the GII.3 cluster (I, II, III, and NC), to the $Le^x$ antigen has been demonstrated (42). Thus, it is necessary to perform more genetic and surveillance studies to determine whether subsets of secretors and nonsecretors share HBGA epitopes for GII.3 or whether certain GII.3 sublineages have gained the ability to bind to both nonsecretors and secretors.

We also documented genetic variation and functional site conservation among major circulating NoV variants, which may contribute to our understanding of the evolution of NoVs. The NoV GII.4 variants 2006b, New Orleans 2009, and Sydney 2012 emerged as major epidemic variants in Vietnam in different seasons, but the amino acids of the HBGA-binding interfaces were conserved across all three strains, including in all three binding sites (amino acids 344 to 347 [TRGH], 374D, and 441 to 443 [SG]) (43). This result is consistent with previous findings on the high levels of amino acid conservation found at the HBGA-binding interfaces among known GII.4 variants (15, 34), indicating that strong selection pressure against genetic variation at these sites is imposed by human HBGAs. However, substitution at epitopes A, D, and F, as defined by Debbink et al. (33), and at amino acids adjacent to sites of interactions with HBGAs could affect the binding affinity of the virus.

Among GII.3 NoV strains, there were no major changes in HBGA binding sites 1 (amino acids 357 to 360 [TRAH]), 2 (386D), or 3 (amino acids 448 to 450 [SSG]) between secretor- and nonsecretor-infecting variants. However, substitution of glycine by aspartic acid (G387E) adjacent to the second HBGA bind-

ing site (amino acids TESG/E) was identified, which could be responsible for the nonsecretor-infecting phenotypes of the GII.3 NoVs. In addition, two amino acid variants (D385G and D385E) were found in strains from nonsecretors, whereas five of the six strains from secretors possessed the D385G substitution. It remains unknown whether this residue (adjacent to the HBGA binding site 386D) is responsible for differences in the HBGA binding patterns. Whether these substitutions are associated with the recently reported increase in NoV GII.3 strains in some regions (42, 44, 45) also remains unknown. A nonsecretor-infecting GII.3 variant in an outbreak in China was recently reported (41).

RV surveillance in Vietnam began in 1998 and is ongoing. In our study, the majority (90%) of RV (and NoV) infections occurred in children younger than 2 years of age, similar to previous findings (39, 40). Over the entire surveillance period, the most common genotype was G1P[8]. Strains belonging to G2P[4], G9, G5P[6], G3, and G12 appeared for short periods in 2000, 2001, 2003 to 2004, 2005, and 2008, respectively; however, the major P types are still P[8], P[4], and P[6], although a small number of P[9] strains have been documented in Vietnam (37, 38, 46, 47). Recently, it has been shown that P[9] RVs bind type A antigen (10). Thus, HBGA binding could be a common factor for all human RVs, and the [P] type could be a major determinant of RV host ranges.

Our study analyzed 260 paired stool-saliva samples, which represented a subset of the total of 407 admissions during the second study period (from September 2011 through September 2012), The NoV and RV detection rates were lower in this subset of samples than in all admissions during this period. However, this reduction in detection rates is unlikely to alter our conclusions, which were based on the virus genotypes rather than virus-positive detection rates.

Our findings on the distributions of different HBGA phenotypes and the relationships of HBGAs to NoV and RV infections in Vietnamese children are significant in terms of future disease control. It is possible that vaccine responses by people with different HBGA types should be evaluated. For example, in a human volunteer challenge study, it was reported that nonsecretors were less likely to mount antibody responses upon infection, due to a lack of interactions between the NoV GII.4 virus and host HBGA receptors (13). The prevalence of the $Le^{a+}$ phenotype in Vietnamese children is similar to that reported for other countries from this region (48, 49). The partial secretor ($Le^{a+b+}$) phenotype observed in Asian populations is quite unique and might be associated with high levels of susceptibility to NoV. A study in Nicaragua showed an absence of this phenotype in both NoV-infected and control groups (50). In addition, that study found that greater proportions of secretors than nonsecretors developed antibodies to NoV and higher antibody titers were induced in secretors than in nonsecretors, indicating that nonsecretors may develop less-robust immune responses following vaccination for the virus (50). In a study in Burkina Faso, which demonstrated an absence of $Le^{a+b+}$ antigens and a high prevalence (32%) of $Le^{a-b-}$ antigens, a strong association between NoV infection and secretor status was identified, with the exception of two cases of GII.4 and GII.7 infections in nonsecretors (35).

In conclusion, we found strong associations between infection by the predominant rotavirus and norovirus genotypes and HBGA secretor status in children. Our study could contribute to future RV and NoV applications and vaccine strategy development.

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